Title

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Journal

Clinical chemistry and laboratory medicine, 58(5)

ISSN

1434-6621

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Publication Date

2020-04-01

DOI

10.1515/cclm-2019-0600

Peer reviewed

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Validation of a liquid chromatography tandem mass spectrometry (LC-MS/MS) method to detect cannabinoids in whole blood and breath

https://doi.org/10.1515/cclm-2019-0600
Received June 13, 2019; accepted August 21, 2019

Abstract

Background: The widespread availability of cannabis raises concerns regarding its effect on driving performance and operation of complex equipment. Currently, there are no established safe driving limits regarding Δ^9-tetrahydrocannabinol (THC) concentrations in blood or breath. Daily cannabis users build up a large body burden of THC with residual excretion for days or weeks after the start of abstinence. Therefore, it is critical to have a sensitive and specific analytical assay that quantifies THC, the main psychoactive component of cannabis, and multiple metabolites to improve interpretation of cannabinoids in blood; some analytes may indicate recent use.

Methods: A liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed to quantify THC, cannabinol (CBN), cannabidiol (CBD), 11-hydroxy-THC (11-OH-THC), (±)-11-nor-9-carboxy-Δ^9-THC (THCCOOH), (+)-11-nor-Δ^9-THC-9-carboxylic acid glucuronide (THCCOOH-gluc), cannabigerol (CBG), and tetrahydrocannabivarin (THCV) in whole blood (WB). WB samples were prepared by solid-phase extraction (SPE) and quantified by LC-MS/MS. A rapid and simple method involving methanol elution of THC in breath collected in SensAbues® devices was optimized.

Results: Lower limits of quantification ranged from 0.5 to 2 μg/L in WB. An LLOQ of 80 pg/pad was achieved for THC concentrations in breath. Calibration curves were linear ($R^2 > 0.995$) with calibrator concentrations within ±15% of their target and quality control (QC) bias and imprecision ≤15%. No major matrix effects or drug interferences were observed.

Conclusions: The methods were robust and adequately quantified cannabinoids in biological blood and breath samples. These methods will be used to identify cannabinoid concentrations in an upcoming study of the effects of cannabis on driving.

Keywords: breath; cannabis; LC-MS/MS; mass spectrometry; THC; whole blood.

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Introduction

Cannabis is available for medicinal purposes in many states. An expert review from the National Academies of Science, Engineering, and Medicine (2017) found that there was conclusive or substantial evidence for the use of cannabis and/or cannabinoids for certain conditions (e.g. chronic pain, emesis, spasticity due to multiple sclerosis), with mixed or limited evidence for many other conditions [1]. Whole-plant-based and non-prescription products still dominate the market, and cannabis as a medicine remains controversial [2]. Furthermore, as more cannabinoid pharmacotherapies are identified, the need for assays to accurately measure cannabinoid concentrations will increase.

Support for recreational legalization of cannabis is growing [3]. With this comes concerns regarding the

impact of acute cannabis use on safety and driving performance. According to the National Roadside Study of Alcohol and Drug Use by Drivers, Δ^9-tetrahydrocannabinol (THC) was the most frequently detected illicit drug in drivers [4]. THC impairs learning [5] and driving-related functions, such as reaction time and tracking ability [6]. This impairment is dose-dependent and can last for several hours [6, 7].

There are several challenges in determining impairment while driving under the influence of cannabis (DUIC). The time between a traffic stop or motor vehicle crash and a blood draw is typically 1.5–4.5 h [8, 9], while THC blood concentrations are >90% cleared within 1.4 h [10]. Therefore, measured THC concentrations likely will not reflect concentrations at the time of the incident. Blood is currently the standard matrix for toxicological interpretations but a non-invasive matrix such as oral fluid or breath may enable more timely roadside collections. In addition, blood THC concentrations do not correlate with cognitive effects [11]. Maximum THC concentrations are typically observed within 15 min of the start of smoking but the subjective "high" and objective impairment can last for several hours [12]. Finally, the clearance rate of THC depends on the frequency of cannabis use. THC can be detected (LLOQ 0.25–0.5 µg/L) for several weeks in the blood of chronic users [13], but only for days in occasional users [14].

Given the tenuous relationship between blood THC concentrations and impairment, it may prove fruitful to examine additional THC metabolites. We thus optimized and validated a liquid chromatography tandem mass spectrometry (LC-MS/MS) method that could simultaneously quantify multiple cannabinoids and metabolites, including THC, 11-hydroxy-THC (11-OH-THC), (±)-11-nor-9-carboxy-Δ^9-THC (THCCOOH), (+)-11-nor-Δ^9-THC-9-carboxylic acid glucuronide (THCCOOH-gluc), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), and tetrahydrocannabivarin (THCV), in whole blood (WB) in a single 5-min run. Furthermore, the detection of THC from breath specimens was validated.

Materials and methods

Chemicals, materials, and blank matrices

Stocks of 1 mg/mL of CBD, THC, CBN, 11-OH-THC, CBG, THCV, THCA-A, and THCCOOH-D_3 as well as 100 µg/mL solutions of THCCOOH, THCCOOH-gluc, CBD-D_3, THC-D_3, CBN-D_3, and 11-OH-THC-D_3 were purchased from Cerilliant (Round Rock, TX, USA). A 10-µg/mL THC-gluc stock was obtained from ElSohly (Oxford, MS, USA).

Optima LC/MS grade acetonitrile, methanol, 2-propanol, formic acid (FA), and ammonium acetate were obtained from Fisher Scientific (Hampton, NH, USA). Oasis PRiME HLB 96-well solid-phase extraction (SPE) plates with 30 mg sorbent/well, 96-well collection plates, quick-load glass inserts, silicone/PTFE-treated 96-well square plug cap mats, and amber max recovery autosampler vials were acquired from Waters (Milford, MA, USA). Borosilicate glass tubes were from Fisher Scientific and 18.2 MΩ-cm water was dispensed from a Siemens PureLab Ultra purification system.

Blank WB was created by mixing acid citrate dextrose (ACD)-packed red blood cells with ABO-compatible plasma and confirming the absence of detectable cannabinoids. Breath matrix consisted of an unused SensAbues® electret filter material ("pad") cut into 28 cm^2 squares to match the intake surface area of the SensAbues® collection device.

Preparation of standards

Calibrators were prepared in methanol using class A glass volumetric pipettes and glass volumetric flasks. All stock and working calibrators were stored at –20 °C.

Whole blood standards: Concentrated methanolic stocks of 10,000 µg/L (lacking THC-gluc) and 1000 µg/L containing CBN, CBD, THC, 11-OH-THC, CBG, THCV, THCA-A, THCCOOH, THCCOOH-glu, and THC-gluc were prepared by mixing and diluting the commercially available standards. Parallel dilutions of the 1000-µg/L stock were used to formulate the remainder of the working standards in methanol. Final concentrations were 10,000 (lacking THC-gluc), 1000, 500, 100, 20, 10, and 5 µg/L. Working standards were added to blank WB at a 1:10 dilution to give final concentrations of 1000, 100, 50, 10, 2, 1, and 0.5 µg/L, respectively.

Breath standards: Parallel dilutions of a 100-µg/mL stock of THC created working standards at concentrations of 10,000, 4000, 100, 20, and 5 µg/L in methanol. To create a calibration curve, 25 µL of the 5-, 20-, 4000-, and 10,000-µg/L standards and 50 µL of the 10,000-µg/L standard were pipetted directly onto unused SensAbues® pads for final amounts of 125, 500, 100,000, 250,000, and 500,000 pg/pad, respectively.

Internal standard (IS): A concentrated deuterium-labeled stock solution was prepared by mixing and diluting THC-D_3, THCCOOH-D_3, CBD-D_3, CBN-D_3, and 11-OH-THC-D_3 in methanol. Final concentrations were 100 µg/L for all compounds except CBD-D_3. CBD-D_3 was added at 10 µg/L because 100 µg/L caused interference, presenting as a shoulder on the CBG qualifier ion peak (Supplementary Figure 1).

The IS for CBN, CBD, THC, 11-OH-THC, and THCCOOH were the corresponding deuterated compounds. CBD-D_3 was the IS for CBG and THCA-A. 11-OH-THC-D_3 was the IS for THC-gluc and THCV. THCCOOH-D_3 was the IS for THCCOOH-gluc due to the presence of a double peak in THCCOOH-gluc-D_3 (Supplementary Figure 2).

IS was added to WB at a 1:10 dilution, resulting in final concentrations of 1 µg/L for CBD-D_3 and 10 µg/L for all others. For breath analysis, only THC was monitored. Twenty-five microliters of IS was spiked into a clean SensAbues® device containing a pad for a final concentration of 250 pg THC-D_3/pad.

Preparation of quality control (QC) material

QC working stocks were made by parallel dilution of a 1000-µg/L stock, as described for the calibrators, except using different lots for all compounds except for THC-gluc where only one lot was commercially available. At least one set of QCs at three different concentrations were included in every batch. The concentration of WB working QCs after validation were 33.3, 100, and 833 µg/L. These were diluted 1:10 into WB for final concentrations of 3.3, 10.0, and 83.3 µg/L, respectively. The concentration of breath QC stocks were 6.0, 60.0, and 6000 µg/L. Pads were spiked with 25 µL for final concentrations of 150, 1500, and 150,000 pg THC/pad, respectively.

Sample collection

Participants provided blood and breath samples as part of a larger study examining cannabis-related driving impairment approved by the University of California, San Diego Human Research Protections Program (IRB # 160641). Consenting volunteers (n = 5) were given a joint containing 13.4% THC procured from the National Institute on Drug Abuse Drug Supply Program. Blood and breath samples were collected prior to and ~10 min after smoking cessation. Venous blood was drawn from the arm in sodium fluoride (NaF) vacutainer tubes. WB was transferred to cryovials and stored at –20 °C for up to 3 months until analysis [15]. Breath samples were collected over a 3-min period using a SensAbues® device containing a pad to trap aerosol breath (AB, Sweden). Mouthpieces were discarded, and the devices containing collection pads were capped and stored at –20 °C for up to 6 months until analysis [16].

Sample processing

Whole blood: Calibrators and QCs were prepared by fortifying calibrators or QCs (20 µL) into 200 µL blank WB in borosilicate tubes. Blank and volunteer WB (200 µL) were added to corresponding tubes and fortified with 20 µL methanol. IS (20 µL) was added to all tubes except one of two blank tubes, resulting in a "blank" and "blank + IS". Proteins were precipitated by adding 500 µL ice-cold acetonitrile with 0.1% FA dropwise into each tube while vortexing. Samples were centrifuged at 1962 × *g* for 10 min. Supernatants were poured into new tubes, diluted with 1.1 mL 18 MΩ water, and loaded onto an SPE plate. SPE was performed using a positive pressure apparatus (Waters Positive Pressure-96). Samples were loaded onto the sorbent material at low pressure (10 psi) for 5 min. Next, samples were washed twice with 500 µL 25% methanol. Samples were eluted twice with 100 µL 90:10 acetonitrile:isopropanol and twice with 100 µL 50:50 acetonitrile:methanol with 2% FA into a 96-well plate with glass inserts. Samples were dried with 40 °C N_2 gas (Porvair Sciences MiniVap), reconstituted in 200 µL 50% acetonitrile with 0.1% FA, and capped with a cap mat. Plates were vortexed (Multi-tube vortexer, Fisher Scientific), centrifuged at 1962 × *g* for 10 min, and loaded into an autosampler.

Breath: A pad was placed inside clean SensAbues® devices for the calibrators, QCs, blank, and blank + IS. These were placed on top of borosilicate tubes with the device neck inside each tube. Devices from volunteers were also placed into tubes. Working IS (25 µL) was added to each device except for the blank. Next, QCs and calibrators were added to the corresponding devices. The devices were dried at room temperature for 10 min. Pads were pushed into the neck of the device with a clean wooden applicator. Samples were eluted twice with 2.5 mL of methanol over 10 min. Residual methanol was squeezed from the device using a pipette bulb affixed on top of each device. Samples were mixed and 1.5 mL was transferred to each of the two max recovery vials (one for processing, one for storage). Vials were dried under nitrogen at 60 °C for 25 min, re-suspended with 200 µL 50% acetonitrile with 0.1% FA, and loaded into the autosampler.

Liquid chromatography (LC)

LC was performed in a similar manner to a previously described method [17]. Briefly, a Waters Acquity i-class UPLC equipped with a column heater (40 °C) was utilized. Full-loop 10 µL injections were separated on a BEH C18 column (2.1 × 50 mm, 1.7 µm particles) with a BEH C18 VanGuard pre-column (2.1 × 5 mm, 1.7 µm particles). Separation was performed with a 0.4 mL/min flow rate using mobile phase A (MPA) consisting of 5 mM ammonium formate with 0.1% FA and mobile phase B (MPB) consisting of acetonitrile with 0.1% FA. The method starts with 50% MPB for 30 s followed by a 3.5-min linear gradient increasing to 90% MPB. The column was washed for 15 s at 90% MPB and then re-equilibrated with initial conditions for 45 s. The total run time was 5 min/sample.

Mass spectrometry (MS)

As described previously [17], the LC system was coupled to a Waters TQS-micro tandem quadrupole MS with an electrospray ionization source. All compounds were analyzed in a positive ionization mode with the exception of THCCOOH-gluc, which required negative ionization. Transition ions were collected by scheduled multiple reaction monitoring using previously described parameters [17]. Concentrations were calculated by dividing quantifier transition ion peak areas by IS peak areas and quantified against the calibration curve included with each batch. A representative chromatogram of all quantifier ions and deuterated ISs from a WB calibrator is displayed in Figure 1.

Method validation

Method validation was done in accordance with the Clinical and Laboratory Standards Institute (CLSI) C62-A guidelines for LC-MS/MS. Experimental design was modified from the CLSI EP05-A3 guidelines for the evaluation of the precision of quantitative measurement procedures.

Sensitivity and detection criteria: The LLOQ was determined as the lowest concentration with an acceptable peak shape, signal-to-noise ratio (S/N ≥ 10), accuracy (% bias) and imprecision (% CV) within 20%, and a quantifier-to-qualifier ratio within 20% of the mean calibrator ratio. Peaks for compounds with a corresponding deuterated IS (THC, 11-OH-THC, THCCOOH, CBN, and CBD) were identified based on a relative RT of 1.01 ± 0.02. Compounds without a corresponding

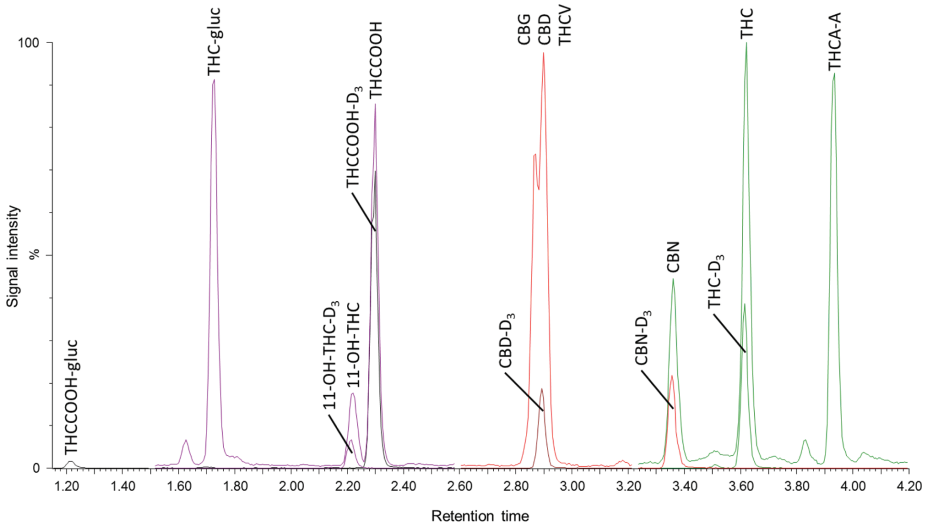

Figure 1: Total ion chromatogram of a 10-µg/L calibrator in whole blood.
Each peak is labeled with the corresponding cannabinoid(s). Signal intensity (y-axis) is plotted against retention time (x-axis). All were chromatographically resolved with the exception of CBG, CBD, and THCV.

deuterated IS were identified by RTs matching within ±0.02 min of the calibrators. An exception to this was THCCOOH-gluc, which exhibited an RT shift of ~0.06 min in biological samples compared to commercially available calibrators due to isomeric differences (Supplementary Figure 2).

Linearity: Calibration curves were established with five to seven calibrators per compound in WB and five calibrators in breath. A line was fitted using a least-squares regression with a 1/x weighting factor. Linearity was validated with five replicates of all calibrators analyzed on different days. Acceptability criteria required that all calibrators except the LLOQ quantify within ±15% of the target concentration; the LLOQ was required to quantify within ±20%. Imprecision criteria for all calibrators was ≤20% CV and the coefficient of determination (R^2) was required to be ≥0.995. For each batch, a maximum of one calibrator was allowed to be excluded from the curve for cause.

QC bias and imprecision: The QC bias and inter-day imprecision were determined using two concentrations of QC each day for 20 days (n = 20 per QC). The intra-day imprecision was assessed with six replicates of two QC concentrations within the same run. Imprecision was required to be ≤20%. QC values were reassigned based on mean concentrations obtained during validation.

Process efficiency and matrix effects: The total process efficiency (PE) was determined by comparing area counts of blank matrix fortified with low QC, and IS prior to sample processing were compared to area counts of "neat" sample containing low QC and IS in the elution solvent [18]. At least three replicates of three concentrations across the analytical measuring range were used for both sets. For WB, this was done in both ACD and NaF blood to determine if calibrators created using ACD blood would accurately quantify analytes in NaF blood.

WB in ACD and NaF tubes and breath samples were collected from 10 drug-free volunteers. The matrix effect was quantified by spiking samples with the low QC prior to processing. Recovered concentrations were then compared to the low QC mean established in validation.

Drug interferences: The blank matrix fortified with low QC was spiked with either methanol or one of five pools containing supra-physiological concentrations of 10 commonly prescribed or abused drugs [17]. Lack of interference was defined as ≤20% bias in the quantification of the low QC concentration in the presence of the different potential interferents.

Autosampler stability and carryover: The stability of samples stored in the autosampler (10 °C) was assessed by comparing the concentrations of QCs and volunteer samples obtained during the initial run and those obtained 24, 48, 72, and 96 h later. Samples were considered stable if the concentration was quantified within 20% of the initial concentration.

Carryover was investigated by injecting a blank + IS before and immediately after the highest calibrator. The blank was visually inspected for any identifiable peak. The criteria for acceptable carryover was a concentration <20% of the lowest calibrator.

Results

Limits of detection, accuracy, and linearity

A representative chromatogram of each reported compound at the LLOQ is shown (Figure 2). The LLOQs, ULOQs, calibration accuracy range, and mean R^2 value are listed in Table 1. LLOQs in WB ranged from 0.5 to 2 µg/L. The LLOQ for THC in breath was 80 pg/pad. THC-gluc and THCA-A in WB did not pass the validation criteria (see following

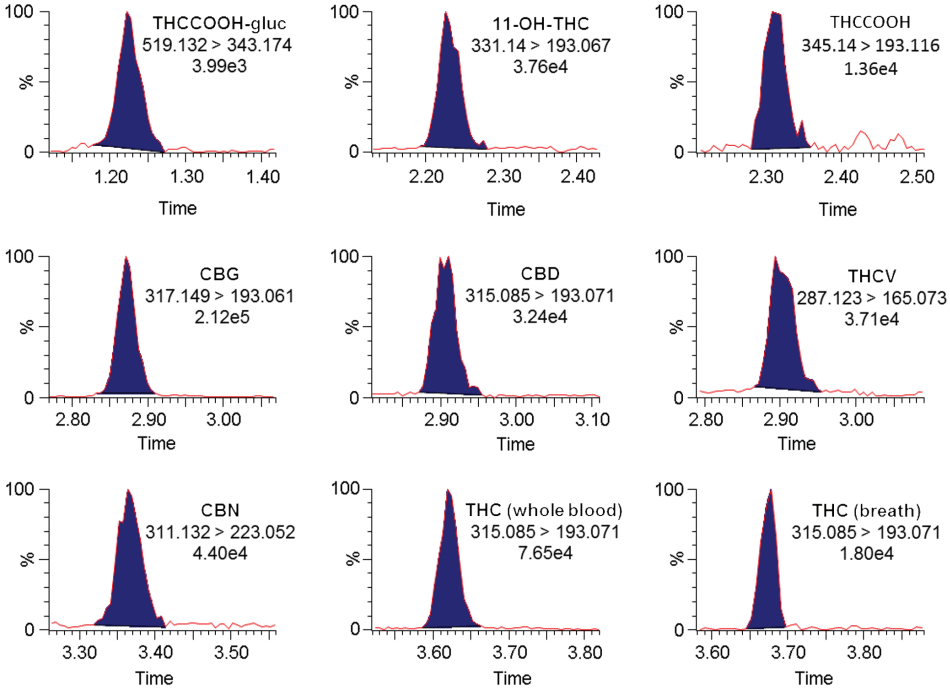

Figure 2: Representative extracted ion chromatograms of the quantifier transition ions for all cannabinoids at the lower limit of quantification.
The percent signal intensity (y-axis) is plotted against retention time (x-axis). The cannabinoids shown, from left to right, include THCCOOH-gluc, 11-OH-THC, THCCOOH, CBG, CBD, THCV, CBN, and THC in whole blood, and THC in breath. The lower limit of quantification ranged from 0.5 to 2 µg/L in blood and was 80 pg/pad in breath.

Table 1: Linearity and limits of quantification.

Cannabinoid	LLOQ, µg/L	ULOQ, µg/L	Calibrator % accuracy range	Mean R^2 of calibration curve
THCCOOH-gluc	2.0	1000	93–108	0.9997
11-OH-THC	1.0	100	88–110	0.9996
THCCOOH	1.0	1000	92–109	0.9997
CBG	1.0	100	90–111	0.9993
CBD	0.5	100	91–110	0.9988
THCV	0.5	100	86–112	0.9990
CBN	0.5	1000	91–114	0.9997
THC (blood)	0.5	1000	83–111	0.9992
THC (breath)	80 pg/pad	500,000 pg/pad	85–114	0.9985

text). For all other compounds, linearity throughout the measurable range was validated.

QC bias and imprecision

All QCs were within 8% of the target concentration except for THCV in WB, which exhibited a 14.8% bias for QC2 (Table 2). The intra-day imprecision ranged from 1 to 8.6% for all cannabinoids. The inter-day imprecision was slightly higher for most compounds and ranged from 4.3 to 12.5%.

Process efficiency

PE was determined in ACD and NaF anticoagulated blood (Table 3). Similar efficiencies were obtained in both blood types with the exception of CBN and THC (>20% difference). Their corresponding ISs showed similar differences between the two blood types and therefore corrected for matrix effects. However, CBN-D_3 and THC-D_3 could not be used as an IS for any other compound due to this issue. Similar total PEs were observed between THC and THC-D_3 in the breath method (Table 3).

Table 2: QC bias and imprecision.

Cannabinoid	Bias, %		Intra-day precision, % CV		Inter-day precision, % CV	
	QC 1	QC 2	QC 1	QC 2	QC 1	QC 2
THCCOOH-gluc	−1.7	−4.9	8.6	4.3	6.8	8.1
11-OH-THC	−5.1	−3.3	4.1	3.1	7.8	5.6
THCCOOH	−2.4	−3.1	5.0	3.6	6.1	5.8
CBG	−6.2	−7.9	4.4	1.0	5.5	12.5
CBD	−5.4	−0.8	3.8	3.0	6.0	4.3
THCV	−5.1	14.8	2.3	4.5	6.0	7.1
CBN	−4.2	−1.1	5.4	2.6	6.5	4.9
THC (blood)	−1.8	1.9	2.4	2.0	6.9	5.2
THC (breath)	−5.1	−7.7	4.7	7.5	8.9	5.0

Target concentrations for whole blood: QC 1 = 3.3 μg/L and QC 2 = 3.3 μg/L. Target concentrations for breath: QC 1 = 150 pg/pad and QC 2 = 1500 pg/pad.

Table 3: Process efficiency for acid citrate dextrose (ACD) blood, sodium fluoride (NaF) blood, and breath.

	Recovery, %		% Difference
	ACD	NaF	(NaF − ACD)
Cannabinoid (blood)			
THCCOOH-gluc	67.9	70.8	2.9
11-OH-THC	67.4	81.0	13.6
11-OH-THC-D_3	59.4	70.2	10.8
THCCOOH	76.1	80.9	4.8
THCCOOH-D_3	65.7	66.0	0.3
CBG	62.1	68.1	6.0
CBD	67.5	77.6	10.1
CBD-D_3	60.7	66.4	5.7
THCV	78.4	86.6	8.2
CBN	39.5	69.0	29.5
CBN-D_3	35.6	61.2	25.6
THC	41.1	79.7	38.6
THC-D_3	33.3	68.4	35.1
Cannabinoid (breath)			–
THC		52.0	–
THC-D_3		61.5	–

Matrix effects and drug interferences

To assess the matrix effect, samples were spiked with 3.3 μg/L QC and the recovered concentration was compared to the expected values (Table 4). All biases were <20% with the exception of THC-gluc (–21%) and THCA-A (53%) in blood. Due to this interference and a lack of deuterated IS to correct for it, THC-gluc and THCA-A failed validation. Breath samples spiked with 150 pg/pad did not show a matrix effect. No drug interferences were observed (Table 5).

Table 4: Quantitative matrix interference between authentic samples and the corresponding blank matrix.

Cannabinoid	Mean % bias
THCCOOH-gluc	6
THC-gluc	−21
11-OH-THC	12
THCCOOH	5
CBG	−1
CBD	7
THCV	17
CBN	9
THC (blood)	8
THCA-A	53
THC (breath)	7

Autosampler stability and carryover

THC concentrations in processed breath remained stable for at least 96 h in the autosampler; all samples were re-quantified within 20% of the initial concentration. In WB, all cannabinoids were stable for at least 24 h. At 48 h post-extraction, all compounds except THCCOOH-gluc were stable. By 72 h, several compounds, including CBN, THC, and THCCOOH-gluc, were not quantifiable. Methanolic calibrators and QCs were stable at –20 °C for at least 2 years.

No carryover was observed for any cannabinoid in either matrix.

Authentic sample quantification before and after smoking

Concentrations of all compounds were measured prior to and after smoking in five volunteers as proof of concept that our method accurately measures THC and its metabolites in authentic samples (Table 6). THCCOOH and THCCOOH-gluc were detectable in WB of four of the five volunteers prior to smoking. After smoking, all cannabinoids except THCV and CBD were detected in WB. All participants had detectable levels of THC in breath after smoking.

Discussion

We describe a robust, validated method for the quantification of eight cannabinoids in WB and THC in breath. The WB method expands on previously published approaches [19–24] and includes additional cannabinoids in a single

Table 5: Drug interferences expressed as percent bias compared to the methanol-spiked QC.

Cannabinoid	Drug pool 1	Drug pool 2	Drug pool 3	Drug pool 4	Drug pool 5
THCCOOH-gluc	8.3	−1.0	−2.8	8.3	2.8
THC-gluc	15.9	5.3	6.8	11.4	9.1
11-OH-THC	9.4	6.5	9.4	9.4	12.5
THCCOOH	2.7	2.2	−8.1	−2.7	−5.4
CBG	16.7	−5.3	16.7	13.3	13.3
CBD	20.0	−3.1	13.3	13.3	13.3
THCV	−2.1	−7.9	−6.3	−8.3	0.0
CBN	6.1	−1.1	6.1	9.1	15.2
THC (blood)	11.8	−2.2	8.8	8.8	8.8
THCA-A	−2.1	−7.9	−12.1	6.1	6.1
THC (breath)	7.9	6.2	8.4	−5.4	6.4

Table 6: Proof-of-concept cannabinoid concentrations before and after smoking a joint containing 13.4% THC.

Cannabinoid	Prior to smoking, µg/L		Ten minutes after smoking cessation, µg/L	
	Median	Range	Median	Range
THCCOOH-gluc	21.8	<LLOQ–275	24.7	<LLOQ–235
11-OH-THC	<LLOQ	<LLOQ–1.8	3.0	1.5–12.1
THCCOOH	9.8	<LLOQ–54.5	26.7	11–64.7
CBG	<LLOQ	<LLOQ	1.1	<LLOQ–1.6
CBD	<LLOQ	<LLOQ	<LLOQ	<LLOQ
THCV	<LLOQ	<LLOQ	<LLOQ	<LLOQ
CBN	<LLOQ	<LLOQ	4.9	1.1–7.3
THC (blood)	<LLOQ	<LLOQ–3.9	60.9	10.3–78.4
THC (breath)	<LLOQ	<LLOQ–242 pg/pad	163,000 pg/pad	88,595–279,000 pg/pad

5-min run. The breath method offers a simplified, yet accurate approach to process and quantify THC in breath. Our method quantifies THC and several cannabis metabolites in authentic human blood and breath samples. Together, these methods will aid in future cannabis therapeutic efficacy and driving safety studies.

There is no agreement in the blood THC concentration that defines impairment. This contrasts with blood alcohol concentrations (BAC), for which concentrations as low as 0.05%–0.08% are strongly correlated with impairment [25]. Portable breath analyzers are used as preliminary screens to estimate BAC. Given the invasive nature of blood draws and frequent collection delays, there is increasing interest in the detection of THC in an easily collected matrix, such as breath. THC concentrations in breath indicate recent use and may correlate with blood concentrations [26]. Furthermore, breath concentrations are correlated with physiological changes such as change in pulse rate and pupil diameter seen after smoking [27]. Therefore, breath could serve as an alternative matrix for roadside impairment assessment.

Other groups published methods to detect cannabinoids in breath. Beck and colleagues offered a method that required a 1-h incubation in a thermostatic bath at 37 °C combined with two extraction procedures [28]. Here, we offered a simplified method that reduced turnaround time. A more recent method using azo coupling derivatization offers sensitive detection with an LLOQ of 0.5 pg THC/mL [29]. Finally, a previously published method using the SensAbues® device measured THC concentrations in breath ranging from 50.7 to 1170 pg/pad at a mean time of 53 min after smoking [16]. We observed much higher concentrations (≥88,595 pg/pad), likely because samples were collected ~10 min after smoking was completed, when THC concentrations in breath would be near maximal. This difference is unlikely due to oral fluid contribution because retention of oral fluid by the trapping baffles integrated into the mouthpiece of the SensAbues® device was previously confirmed by the lack of α-amylase in samples eluted directly from the pad while kept inside the device [30]. Our goal was to validate a method with a quicker and simpler procedure that accurately quantifies THC in breath. Our method offers a straightforward

extraction protocol that does not require disassembling SensAbues® breath devices or derivatization while still achieving good analytical sensitivity.

During validation, THCA-A and THC-gluc exhibited significant matrix effects. Despite this, others have quantified THCA-A [31] and THC-gluc [32] using LC-MS/MS in WB. Sorensen and colleagues removed interfering phospholipids by filtration using a hybrid SPE-phospholipid plate containing a stationary phase with bonded zirconia and successfully quantified THCA-A [31]. Disposable pipette extraction, instead of SPE, was previously used to extract and quantify THC-gluc in WB [32].

We noted an RT shift in THCCOOH-gluc peaks between human samples and calibrators during validation (Supplementary Figure 2). Biological THCCOOH-gluc eluted at 1.28 min and the analytical standard at 1.22 min. The THCCOOH-gluc-D_3 available from Cerilliant (T-080, (±)-cis-11-Nor-9-carboxy-Δ9-THC-D3-glucuronide) produced two isomeric chromatographic peaks. When THCCOOH-gluc-D_3 was base hydrolyzed, it resulted in the formation of THCCOOH-D_3 with a shifted RT compared to the THCCOOH-D_3 standard. However, when THCCOOH-gluc was hydrolyzed, the resulting RT of THCCOOH matched its respective standard. Therefore, the biological form of THCCOOH-gluc is likely a different isomer than the commercially available form [33].

The WB and breath methods developed here facilitate efficient detection of multiple cannabinoid metabolites. It will be used in randomized placebo-controlled trials examining the effect of cannabis on driving performance and improving the detection of impaired drivers as well as other studies.

Acknowledgments: The authors would like to thank Waters Corporation for their support, training, and technical assistance throughout this project.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: This work was supported by the State of California via the Medical Marijuana Regulation and Safety Act (Assembly Bill 266). Additional support was provided by the Center for Medicinal Cannabis Research at the University of California, San Diego, which receives support from the Control, Regulate, and Tax Adult Use of Marijuana Act (California Proposition 64) and philanthropic gifts from the Wholistic Research and Education Foundation.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/cclm-2019-0600).